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Direct Conversion of Resin-Bound Peptides to C-Terminal Esters

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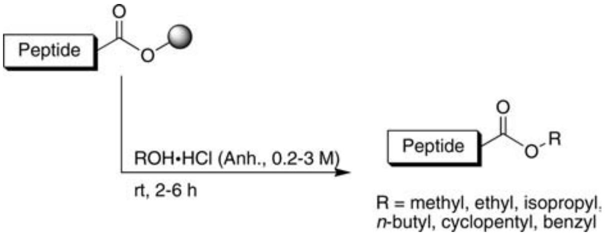
ABSTRACT



A mild and effective method was developed to convert peptides immobilized on the 2-chlorotrityl and Wang resins directly to C-terminal esters. After conventional Fmoc peptide synthesis, treatment with anhydrous HCl (0.2−3 M) in a variety of alcohols was shown to produce the corresponding peptide esters in good yield and purity. Under the mildest conditions investigated, acid-sensitive protection groups such as *N*-Boc, trityl, *tert*-butyl ether, *tert*-butyl ester, and Pbf remain intact.

The development of peptides as therapeutic agents is on the rise.[1] Despite their suboptimal pharmacokinetic properties, peptides demonstrate unparalleled potency and specificity against a wide array of biological targets.[2−6] The increasing importance of peptides in drug discovery has produced a need for high-throughput techniques that introduce the chemical modifications known to improve the pharmacological properties of small molecule drugs.

One approach to improving membrane permeability of drugs containing pharmacologically important carboxylic acids is to mask the hydrophilic moiety as a alkyl ester.[7−9] Upon membrane permeation, the ester prodrug is hydrolyzed by intracellular esterases revealing the active carboxylic acid.[10−12] Applying this approach to the synthetic plan for a peptide library may serve to improve the bioassay hit ratio. However, a survey of the literature produced no viable options for methods with which to convert resin bound peptides directly into C-terminal methyl esters.

The few reported transesterifying cleavage methods generally require long reaction times and/or harsh conditions. For example, one of the earliest methods used methanol or ethanol under strongly basic conditions and required reaction times of 2−24 h.[13−17] Alternately, potassium cyanide was used to promote transesterification with benzyl alcohol from

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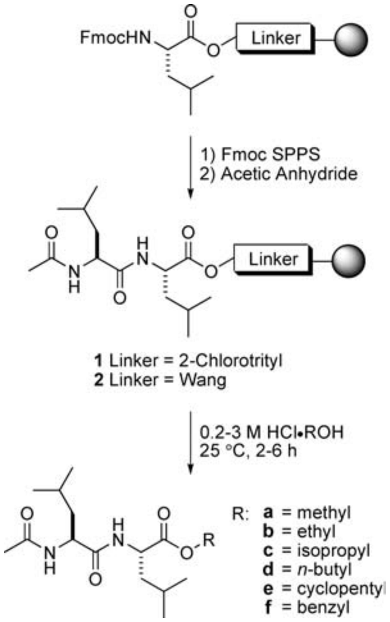
Scheme 1. Fmoc SPPS of the *N*-Acyl-Leu-Leu Dipeptide Used in the Optimization of Cleavage/ Esterification Procedure



the Merrifield resin at elevated temperatures.[18] More recent efforts to facilitate C-terminal modifications, in general, have involved the use of specialized linkers and SPPS in the N to C direction.[19−21] To date, however, there have been no reports of a transesterifying cleavage protocol that yields pure and selectively protected peptides under mild conditions.

Methyl esters have been formed in solution by treating a carboxylic acid with anhydrous methanolic HCl prepared through the reaction between methanol and acetyl chloride.[22,23] The procedure is usually high yielding, and purification of ester products can be as simple as evaporating the methanolic HCl solution. These attributes led us to investigate this reagent in the one-pot cleavage and esterification of dipeptide model compounds **1** and **2** from the 2-chlorotrityl and Wang linkers, respectively (Scheme 1).

A methanolic HCl solution was prepared by the slow addition of acetyl chloride to cold methanol. This solution was stirred for 2 h before being added to aliquots of **1** and **2** that were pre-swelled with minimal methylene chloride. Generally, 20−100 mg of resin was swelled in 0.5−1 mL of methylene chloride, and 3−4 mL of methanolic HCl was added. The cleavage and concomitant conversion to methyl ester was complete within 2−5 h and gave yields of 95% or greater (Table 1). The efficacy of the method with other alcohols was investigated and showed good to excellent results with both primary and secondary alcohols, such as ethanol, 2-propanol, *n*-butanol, cyclopentanol, and benzyl alcohol. In general, conversion to ester was greater than 95%, with yields ranging from 73−99%. The workup of these peptide esters entailed evaporation of the cleavage solution, followed by trituration of the peptide esters with hexanes and water.

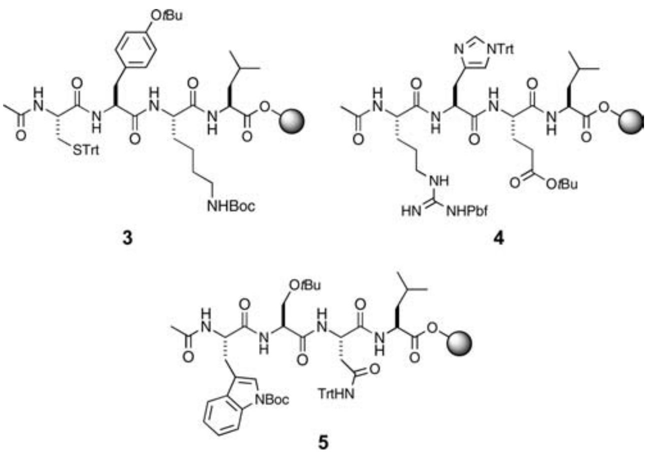


Figure 1. Peptides used to investigate the stability of acid-sensitive protecting groups during cleavage/esterification.

Typically, peptides are cleaved from 2-chlorotrityl and Wang-type linkers by treatment with TFA, frequently resulting in the removal of some or all acid-sensitive side chain protection groups. If a postcleavage side chain modification is required, such as the installation of a fluorophore or a saccharide, C-terminal esterification prior to side chain deprotection may be necessary. Transesterification reactions using peptides **3**−**5** synthesized on the 2-chlorotrityl linker (Figure 1) were performed with HCl concentrations ranging from 0.2−3 M, in order to determine the lowest concentration of HCl that would afford both complete C-terminal esterification and preservation of side-chain protection groups such as N-Boc, *tert*-butyl ester, *tert*-butyl ether, trityl, and pbf.

Using a 0.2 M methanolic HCl solution, peptides **3**−**5** showed good to excellent conversion to C-terminal methyl ester within 3 h. The reaction resulted in good yields of 73−98% with only minor loss of the tyrosine *tert*-butyl ether and the histidine trityl group (Table 2, entries 4−6). The same peptides synthesized on the Wang resin transesterified very slowly with a 0.2 M HCl solution, resulting in low yields of protected peptides with a 2 h reaction time. A longer reaction time improved the yield of the peptide ester but also resulted in the loss of side-chain protection groups.

Higher concentrations of HCl (3 M) enabled removal of the particularly acid-sensitive side chain protection groups such as boc, *tert*-butyl ether, and the trityl groups from cysteine and asparagine. The histidine trityl and arginine Pbf

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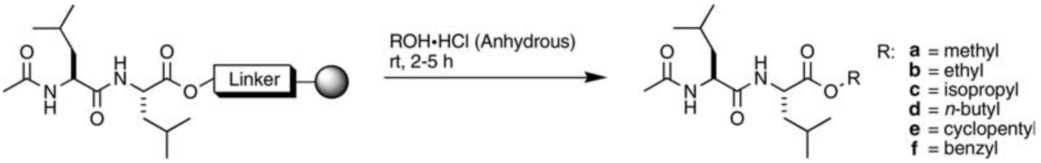
Table 1. Results from Investigation into Scope of Cleavage/Esterification Procedure



peptide + linker	linker	R	HCl (M)	time (h)	% convn[a]	% yield[b]
1a	2-chlorotrityl	methyl	3	2	98	97
1b	2-chlorotrityl	ethyl	3	2	99	99
1c	2-chlorotrityl	isopropyl	3	3	99	80
1d	2-chlorotrityl	*n*-butyl	3	2	98	99
1e	2-chlorotrityl	cyclopentyl	3	3	99	96
1f	2-chlorotrityl	benzyl	3	2	99	99
2a	Wang	methyl	3	5	99	98
2b	Wang	ethyl	3	5	97	91
2c	Wang	isopropyl	3	5	95	78
2d	Wang	*n*-butyl	3	5	98	94
2e	Wang	cyclopentyl	3	5	98%	73%
2f	Wang	benzyl	3	5	99%	89%

[a] Percent conversion was determined by integration of peaks in the UV trace ($\lambda = 198$ nm). [b] Yields of peptides purified by trituration with hexanes and water.

Table 2. Investigation into the Stability of Acid-Sensitive Side-Chain Protection Groups in the Cleavage/ Esterification Reaction with Methanol•HCl and *t*-Butyl Alcohol•HCl (3 h)

entry	peptide	sequence	linker	ROH•HCl (M)	% PG loss[a]	% convn[b]	% yield[c]
1	3	Ac-C(Trt)-Y(*t*-Bu)-K(Boc)-L	Wang	methanol (0.2 M)	10[d]	>99	6
2	4	Ac-R(Pbf)-H(Trt)-E(*t*-Bu)-L	Wang	methanol (0.2 M)	0	>99	8
3	5	Ac-W(Boc)-S(*t*-Bu)-N(Trt)-L	Wang	methanol (0.2 M)	0	>99	5
4	3	Ac-C(Trt)-Y(*t*-Bu)-K(Boc)-L	2-chlorotrityl	methanol (0.2 M)	8[d]	82	74
5	4	Ac-R(Pbf)-H(Trt)-E(*t*Bu)-L	2-chlorotrityl	methanol (0.2 M)	4[e]	77	73
6	5	Ac-W(Boc)-S(*t*-Bu)-N(Trt)-L	2-chlorotrityl	methanol (0.2 M)	0	98	98
7[f]	3	Ac-C(Trt)-Y(*t*-Bu)-K(Boc)-L	2-chlorotrityl	methanol (0.2 M)	14[d]	62	53
8[f]	4	Ac-R(Pbf)-H(Trt)-E(*t*-Bu)-L	2-chlorotrityl	methanol (0.2 M)	35[e]	50	31
9[f]	5	Ac-W(Boc)-S(*t*-Bu)-N(Trt)-L	2-chlorotrityl	methanol (0.2 M)	2[g]	77	74
10	3	Ac-C(Trt)-Y(*t*-Bu)-K(Boc)-L	2-chlorotrityl	*tert*-butyl alcohol (0.2 M)	0	n/a	74
11	4	Ac-R(Pbf)-H(Trt)-E(*t*-Bu)-L	2-chlorotrityl	*tert*-butyl alcohol (0.2 M)	0	n/a	92
12	5	Ac-W(Boc)-S(*t*-Bu)-N(Trt)-L	2-chlorotrityl	*tert*-butyl alcohol (0.2 M)	0	n/a	80

[a] Determined by integration of peaks in UV traces ($\lambda = 198$ nm). [b] Percent conversion to ester was determined by integration of peaks in the UV trace ($\lambda = 198$ nm). [c] Yields of fully protected methyl esters were determined by integration of peaks in the UV trace ($\lambda = 198$ nm) and comparison of product ratios to the mass of peptide obtained. [d] Loss of *t*-Bu ether from tyrosine. [e] Loss of trityl group from histidine. [f] Two-step cleavage and esterification procedure (see the Supporting Information for experimental details). [g] Loss of boc from tryptophan.

groups were considerably more robust and required longer reaction times of 6−12 h for removal. High concentrations of HCl promoted the transesterification of a *tert*-butyl ester, though this problem can be circumvented, in principle, by deprotecting side chain esters after the cleavage and C-terminal esterification reaction is performed at a lower concentration of HCl.

We found that when peptides **3**−**5** were cleaved from the 2-chlorotrityl resin with AcCl in *tert*-butyl alcohol (0.2 M), peptides were obtained as the carboxylic acid in good yield with all protecting groups intact (Table 2). The efficient cleavage and absence of esterification under these conditions may be due to the formation of acetic acid in *tert*-butyl alcohol by way of elimination. Similarly mild conditions are known that effect cleavage from the 2-chlorotrityl resin with preservation of protecting groups (e.g., 1% TFA/DCM).

There are some noteworthy advantages to the one-pot transesterification procedure described above. Synthesis of peptide libraries requires reactions that are high yielding and promote diversity among library members. The procedure described here is effective with both primary and secondary alcohols and is sufficiently mild as to enable the synthesis of a variety of alkyl esters in a 96- or 384 well-plate format utilizing robotic liquid handling. We also found that the one-pot procedure was superior to a two-pot procedure with respect to both efficiency and the ability to obtain consistently high yields of protected peptide esters. A two-pot procedure entailing cleavage of the peptide from the 2-chlorotrityl resin with 1% TFA and subsequent esterification with 0.2 M HCl•methanol (Table 2, Supporting Information) was a considerably longer process and made the management of side-chain protection groups a challenge. In addition, lower concentrations of HCl resulted in incomplete conversion to peptide ester, requiring longer reaction times that led to loss of side-chain protection groups.

We investigated C-terminal racemization in the methanolic HCl cleavage/esterification method by reacting both C-terminal epimers of the AcN-Leu-Leu dipeptide **1** with 3 M methanolic HCl. Optimized LCMS conditions enabled separation of a mixture of the two epimers on a C18 column. We followed the reactions of the individual stereoisomers for 6 h and saw no evidence of C-terminal racemization (see Figure 28 in the Supporting Information).

The transesterifying SPPS cleavage method described above represents a significant improvement over other similar techniques reported previously. It employs commonly used SPPS resins, involves shorter reaction times and milder reaction conditions, and produces higher yields. This method is adaptable and enables the synthesis of peptide esters from a number of primary and secondary alcohols. Furthermore, the concentration of HCl in the cleavage solution can be optimized to selectively preserve or remove a number of acid-sensitive side-chain protection groups. This method offers a promising tool for the high-throughput synthesis of C-terminal peptide ester libraries.

Supporting Information Available: Detailed synthetic procedures; method for calculating product ratios; ^{1}H and ^{13}C NMR spectra and LCMS traces for compounds **1a**−**f** and **2a**−**f**. This material is available free of charge via the Internet at http://pubs.acs.org.

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